

Original Research Article

Immunomodulatory, antiglycation and anti-ulcerative properties of *Ruellia squarrosa* Fenzl Acanthaceae

Khurram Afzal¹, Muhammad Uzair¹, Bashir Ahmad Chaudhry¹, Samina Afzal¹, Ardas Masood², Malik Saadullah¹ and Muhammad Imran Qadir^{2*}

¹Faculty of Pharmacy, ²Institute of Molecular Biology & Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

*For correspondence: **Email:** mrirranqadir@hotmail.com

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Abstract

Purpose: To evaluate the immunomodulatory, antiglycation and anti-ulcerative properties of *Ruellia squarrosa* Fenzl. Acanthaceae.

Methods: Aerial parts and roots of *Ruellia squarrosa* were collected and extracted by maceration using dichloromethane and methanol as solvents. Luminol-enhanced chemiluminescence assay was used to evaluate immunomodulatory activity while antiglycation assay was performed by fluorescence method with rutin as standard. Anti-ulcerative activity was evaluated by enzymatic methods, namely, urease inhibition and carbonic anhydrase inhibition assays.

Results: Dichloromethane extract showed immunomodulatory activity with half-maximal inhibitory concentration (IC_{50}) of 39.48 ± 8.06 % using ibuprofen as standard and antiglycation effect ($IC_{50} = 382.21 \pm 3.43$) using rutin as standard. The methanol extract of the aerial parts of the plant showed urease inhibition activity ($IC_{50} = 130.2 \pm 0.57$) using thiourea as standard. The methanol extract of the aerial parts of the plant also showed carbonic anhydrase inhibition activity ($IC_{50} = 1656.7 \pm 0.08$) using acetazolamide as standard.

Conclusion: It was concluded from the present study that aerial and root extracts of the *Ruellia squarrosa* have significant immunomodulatory, antiglycation and anti-ulcerative properties.

Keywords: *Ruellia squarrosa*, Immunomodulatory, Antiglycation, Anti-ulcerative activity, Carbonic anhydrase inhibition, Urease

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INTRODUCTION

Use of plants started with the start of human civilization, not only for food purpose, but also for healing. The plant materials initially took the form of crude drugs such as poultices, powders, tinctures, and many other herbal formulations [1]. According to WHO, almost 80% of the world's populations depends upon natural products for their health [2]. Glycation is involved in several diabetes-associated complications like retinopathy, neuropathy, and nephropathy. The accumulation of glycation end products along

with proteins compromises their physiological functions [3]. The formation of advanced glycation end-products (AGEs) progressively increases with normal aging and is accelerated in diabetes [4]. Organic material of plant origin with significant antiglycation activity can be used for prevention of diabetic complications. Large amounts of reactive oxygen species (ROS) are produced by neutrophils and monocytes in response to infection which kill microorganisms. Excessive ROS concentration is toxic to host cells that causes tissue damage and numerous inflammatory reactions. Plants that contain

immunomodulatory constituents may be used to reduce such inflammatory responses [5]. Urease catalysis the hydrolysis of urea to form ammonia and carbon dioxide; and therefore urease-inhibitory activity has been designed to quantify the end products produced during this reaction. The advantageous objective of urease inhibition is to reduce the concentration of carbon dioxide and ammonia and ultimately reduction in ulcer. Carbonic anhydrase inhibition is also correlated with anti-ulcerative activity.

Many plants of genus *Ruellia* have been used as diuretic, anti-diabetic, anti-pyretic, analgesic, anti-oxidant, anti-hypertensive and gastro-protective [6,7]. Phytochemical constituents which have a wide range of biological activities such as glycoside, alkaloids, flavonoids, triterpenoids have been reported from genus *Ruellia* [8, 9]. *Ruellia squarrosa* has recently been evaluated for its anticancer activity [10].

This study was designed to evaluate the immunomodulatory, antiglycation and anti-ulcerative enzyme inhibition activities of dichloromethane and methanol extracts of aerial parts and roots of *Ruellia squarrosa*.

EXPERIMENTAL

Ruellia squarrosa was collected from the area near to Bahauddin Zakariya University, Multan. Dr. Altaf Ahmed Dasti, from the Institute of Pure and Applied Biology, B.Z.U, Multan identified the plant. The plant parts were deposited in the department's herbarium with the specimen's No FL.C. 1-2 for future reference. The shaded and dried plant material (roots and aerial parts) of *Ruellia squarrosa* was ground and macerated with dichloromethane and methanol for three days. Both dichloromethane and methanol extracts were concentrated by using the Rota Vapor. The four extracts used in this study were: RSAD (*Ruellia squarrosa*-Aerial parts-Dichloromethane extract); RSAM (*Ruellia squarrosa*-Aerial parts-Methanol extract); RSRD (*Ruellia squarrosa*-Roots-Dichloromethane extract); and RSRM (*Ruellia squarrosa*-Roots-Methanol extract).

Immunomodulatory activity

We estimated the immunomodulatory activity of the extracts by using Luminol-enhanced chemiluminescence assay. Fresh blood was taken from healthy human volunteers. 25 μ L of whole blood and 25 μ L of the sample in Hank's balanced salt solution (containing $MgCl_2$ and $CaCl_2$) were placed in an incubator. Then the mixture was dissolved in MeOH (3.1–100 μ g/mL)

and added 25 μ L of zymosan, followed by chemiluminescence probe luminol (25 μ L). Then HBSS++ was added to adjust the final volume to 0.1 ml. The chemiluminescence's peaks were recorded with a Lumino meter (Luminoskan, Finland) [11].

Antiglycation assay

Bovine serum albumin (BSA) solution was prepared in 20 μ L, 100 mM phosphate buffer with 20 μ L, 3 mM solution of sodium azide. The same concentration was used to prepare glucose solution (800 mM). 20 μ L, 500 mM of dimethyl sulfoxide, 50 μ L of BSA, 50 μ L of glucose, 80 μ L of phosphate buffer were mixed in the well and in the reaction mixture was placed in the incubators at 37 °C for one week. The sample analysis was performed by using spectrophotometer at 440 nm wavelength. [12]. Percentage inhibition of AGEs (advanced glycation end-products) formation was calculated by using Eq 1.

$$\% \text{ Inhibition of AGE formation} = [1 - (F_t / F_c)] \times 100 \dots\dots\dots (1)$$

Anti-ulcerative enzyme inhibition activities

i) Urease inhibition assay: Reaction mixtures comprising 25 μ L of enzyme solution and 55 μ L of buffers were placed in an incubator with 5 μ L of the extract for 15-20 minutes in 96-well plates. Urease inhibition activity was determined by the indophenol method by using spectrophotometer and taking absorbance at 625 nm. Thiourea was used as the standard inhibitor of urease [13].

Percentage inhibition was measured by using formula given in Eq 2.

$$\% \text{ Inhibition} = 100 - (OD_{\text{testwell}} / OD_{\text{control}}) \times 100 \dots (2)$$

ii) Carbonic anhydrase inhibition activity: Carbonic anhydrase inhibition assay was performed according to Ashiq *et al* with slight modification. The total mixture volume was 200 μ L in a well containing 140 μ L (20 Mm HEPES (Bioworld: Cat# 40820000-1) tris buffer (Invitrogen: Cat# 15504-020, pH 7.4), 20 μ L of the enzyme (Sigma Aldrich, C2624, PCode: 1001584424) (0.1 - 0.2 mg/ mL in deionized water); 20 μ L (0.5 mg/mL in DMSO) of test compound was mixed and incubated at 25 °C for 15 min. After incubation pre-read was taken at 400 nm and 20 μ L of substrate (4-nitrophenol acetate, Sigma Aldrich, N8130, lot#BCBK4587V) (0.7 mM in methanol) was added and re-incubated at the same condition for 30 min and after read was taken at 400 nm. Acetazolamide

was taken as positive control. IC_{50} was calculated by making serial dilution of original concentration [14]. Inhibition (H) was calculated as given in Eq 3.

$$H (\%) = 100 - (A_t/A_c)100 \dots\dots\dots (3)$$

Statistical analysis

Data collected were analysed using descriptive statistics. Comparison of means was achieved using one way ANOVA. At 95% confidence interval, 2-tailed p values less than 0.05 were considered significant.

RESULTS

The dichloromethane extract of aerial parts of the plant showed immunomodulatory activity with

IC_{50} 39.48 ± 8.06 using ibuprofen as standard (Table 1).

The dichloromethane extract of the aerial parts of the plant showed antiglycation activity (IC_{50} 382.21±3.43) as compared to standard, rutin (IC_{50} 121.68±1.04); while the other extracts of the plant showed no activity (Table 2).

The methanol extract of the aerial parts of the plant showed urease inhibition activity with IC_{50} 130.2±0.57 using thiourea as standard (Table 3).

The methanol extract of the aerial parts of the plant showed carbonic anhydrase inhibition activity with IC_{50} 1656.7 ± 0.08 using acetazolamide as standard (Table 4).

Table 1: Immunomodulatory activity of dichloromethane and methanol extracts of *Ruellia squarrosa*

Sample	Immunomodulatory		
	Concentration (mM) [*]	Inhibition (%)	IC_{50} ^{**} (μ M, mean±SEM)
RSAM	25	16.57	-
RSRD	-	-0.93	-
RSRM	-	-0.07	-
RSAD	-	0.01	39.48±88.06
Ibuprofen (standard)	25	3.2	11.2±11.9

IC_{50} = half-maximal inhibitory concentration; RSAD (*Ruellia squarrosa*- dichloromethane aerial extract); RSAM (*Ruellia squarrosa*- methanol root extract); RSRD (*Ruellia squarrosa*-roots-dichloromethane extract); and RSRM (*Ruellia squarrosa* methanol root extract)

Table 2: Antiglycation activity of dichloromethane and methanolic extracts of *Ruellia squarrosa*

Sample	Antiglycation		
	Concentration (mg/mL)	Inhibition (%)	IC_{50} (mg/mL, mean±SEM)
RSRD	2	18.28	NA
RSRM	2	39.18	NA
RSAD	2	65.00	382.21±3.43
RSAM	2	44.48	NA
Rutin	2	75.4	121.68±1.04

IC_{50} = half-maximal inhibitory concentration; RSAD (*Ruellia squarrosa*- dichloromethane aerial extract); RSAM (*Ruellia squarrosa*- methanol root extract); RSRD (*Ruellia squarrosa*-roots-dichloromethane extract); and RSRM (*Ruellia squarrosa* methanol root extract)

Table 3: Urease inhibitor activity of dichloromethane and methanol extracts of *Ruellia squarrosa*

Sample	Urease inhibitor activity		
	Concentration (mM)	Inhibition (%)	IC_{50} (μ M, mean±SEM)
RSRD	0.2	29.8	NA
RSRM	0.2	43.1	NA
RSAD	0.2	13.9	NA
RSAM	0.2	61.9	130.2±0.57
Thiourea	0.5	99.8	21.2±1.3

IC_{50} = half-maximal inhibitory concentration; RSAD (*Ruellia squarrosa*- dichloromethane aerial extract); RSAM (*Ruellia squarrosa*- methanol root extract); RSRD (*Ruellia squarrosa*-roots-dichloromethane extract); and RSRM (*Ruellia squarrosa* methanol root extract)

Table 4: Carbonic anhydrase inhibition activity of dichloromethane and methanolic extracts of *Ruellia squarrosa*

Code	Conc. (5mg/mL)	Inhibition (%)	IC ₅₀ (µg/mL, mean±SEM)
RSRD		23±0.07	-
RSAM		65±0.07	1656.7±0.08
RSRM		43±0.02	-
RSAD		56±0.08	1666.6±0.04
Acetazolamide	0.1 µMOL	98±0.56	0.031±0.01

All the samples were dissolved in DMSO. IC₅₀ = half-maximal inhibitory concentration; RSAD (*Ruellia squarrosa*- dichloromethane aerial extract); RSAM (*Ruellia squarrosa*- methanol root extract); RSRD (*Ruellia squarrosa*-roots-dichloromethane extract); and RSRM (*Ruellia squarrosa* methanol root extract)

DISCUSSION

Immunomodulators are chemicals that up-regulate or down-regulate the immune system. Immunomodulators have been accepted as very useful drugs because of their wide functions [15]. Various disease conditions such as infections, organ transplant rejection, cancer, rheumatoid arthritis, and systemic lupus erythematosus are currently treated with immunomodulating agents [16]. Many plants with immunomodulatory activities have been reported e.g. *Thamnia vermicularis* [17]. In this study, aerial parts of the methanol extract of *R. squarrosa* showed a very good immunomodulatory activity, five times greater than the standard.

Glycation is a condensation reaction between sugars and amino groups of proteins and form advanced glycation products (AGPs). This reaction depends on half-life of the proteins and permeability to free glucose [18,19]. AGEs play a useful role in diabetic care where it deposits under endothelial cells and actually causing the decrease in blood sugar concentration. Glycooxidation causes protein dysfunction suggesting the pathological factor involved in diabetes [20,21]. In the present study, dichloromethane extract of aerial parts of *R. squarrosa* showed comparable results with the standard.

Urease and carbonic anhydrase inhibition are correlated with anti-ulcerative activity. Our extracts showed very good anti-ulcerative activity.

CONCLUSION

The findings of this study demonstrate that *Ruellia squarrosa* possesses immunomodulatory

activity and therefore have anti-angiogenic properties and thus, may be suitable for the treatment of cancer. Its antiglycation and anti-ulcerative properties indicate that they could find use in the treatment of diabetes and gastric ulcer, respectively. However, further studies are required to ascertain its clinical potentials.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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